

Nerve excitability changes in critical illness polyneuropathy

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Patients in intensive care units frequently suffer muscle weakness and atrophy due to critical illness polyneuropathy (CIP), an axonal neuropathy associated with systemic inflammatory response syndrome and multiple organ failure. CIP is a frequent and serious complication of intensive care that delays weaning from mechanical ventilation and increases mortality. The pathogenesis of CIP is not well understood and no specific therapy is available. The aim of this project was to use nerve excitability testing to investigate the changes in axonal membrane properties occurring in CIP. Ten patients (aged 37–76 years; 7 males, 3 females) were studied with electrophysiologically proven CIP. The median nerve was stimulated at the wrist and compound action potentials were recorded from abductor pollicis brevis muscle. Strength–duration time constant, threshold electrotonus, current–threshold relationship and recovery cycle (refractoriness, superexcitability and late subexcitability) were recorded using a recently described protocol. In eight patients a follow-up investigation was performed. All patients underwent clinical examination and laboratory investigations. Compared with age-matched normal controls (20 subjects; aged 38–79 years; 7 males, 13 females), CIP patients exhibited reduced superexcitability at 7 ms, from $-22.3 \pm 1.6\%$ to $-7.6 \pm 3.1\%$ (mean \pm SE, $P \approx 0.0001$) and increased accommodation to depolarizing ($P < 0.01$) and hyperpolarizing currents ($P < 0.01$), indicating membrane depolarization. Superexcitability was reduced both in patients with renal failure and without renal failure. In the former, superexcitability correlated with serum potassium ($R = 0.88$), and late subexcitability was also reduced (as also occurs owing to hyperkalaemia in patients with chronic renal failure). In patients without renal failure, late subexcitability was normal, and the signs of membrane depolarization correlated with raised serum bicarbonate and base excess, indicating compensated respiratory acidosis. It is inferred that motor axons in these CIP patients are depolarized, in part because of raised extracellular potassium, and in part because of hypoperfusion. The chronic membrane depolarization may contribute to the development of neuropathy.

Keywords: axonal excitability; intensive care; membrane potential

Abbreviations: BE = base excess; CIP = critical illness polyneuropathy; CMAP = compound muscle action potential; Er = resting potential; Ek = potassium equilibrium potential; SNAP = sensory nerve action potential

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Introduction

Critical illness polyneuropathy (CIP) is a predominantly distal, motor and sensory axonal polyneuropathy, affecting 30–70% of critical care patients (Berek *et al.*, 1996; Leijten *et al.*, 1996; Hund, 2001; Bolton, 2005; Latronico *et al.*, 2005). It is often revealed by failure of weaning from artificial ventilation and contributes to mortality, rehabilitation problems and subsequent impaired quality of life (Zochodne *et al.*, 1987). CIP is strongly associated with systemic inflammatory response

syndrome and multiple organ dysfunction syndrome, but its aetiology is not well understood (Bolton, 1996). CIP has been attributed to either the build-up of neurotoxic factors in the blood or the rapid depletion of essential factors, or to endoneurial hypoxia caused by oedema and reduced microvascular blood flow (Druschky *et al.*, 2001).

Nerve excitability testing is a non-invasive approach to investigating the pathophysiology of peripheral nerve

disorders, which determines the electrical properties of the nerve membrane at the site of stimulation. A recently developed computer-controlled excitability testing protocol measures multiple excitability parameters within 10 min which provide information about the axonal membrane potential, ion channels and ionic environment (Kiernan *et al.*, 2000). For example, the technique has revealed membrane potential changes in patients with chronic renal failure, and with multifocal motor neuropathy, that could not be detected by conventional nerve conduction studies (Kiernan *et al.*, 2002a, b). Nerve excitability testing is therefore a highly appropriate tool for investigating CIP, in which membrane depolarization is a likely aetiological factor, whether caused by the toxic action of cytokines, anaemia or endoneurial hyperkalaemia.

The present study was therefore undertaken to test the hypothesis that axons in CIP patients are depolarized and to compare abnormalities in excitability parameters with putative contributors to nerve damage, to improve our understanding of the pathophysiology and aetiology of this condition and ultimately to help reduce its incidence.

Methods

Recordings were made on 10 patients (aged 37–76 years; mean: 62.2 years; 7 males, 3 females) from the intensive care unit of two major London teaching hospitals. No patient had a history of pre-existing peripheral neuropathy or myopathy or suffered from a disease, such as diabetes mellitus, that is associated with polyneuropathy or myopathy. All patients suffered from CIP. The presence of CIP was assessed by clinical and electrophysiological evaluation. The patients were compared with 20 healthy control subjects (NC) with a similar age range (aged 38–79 years; mean: 58 years; 7 males, 13 females). (Blood gas and bicarbonate measurements were not available for 10 subjects, but the smaller control group used for comparing these parameters was otherwise similar to the larger group, aged 38–78 years; mean: 55.6 years; 3 males, 7 females.) The study was approved by the St Thomas' Hospital Research Ethics Committee and by the National Hospital for Neurology and Neurosurgery and the Institute of Neurology Joint Research Ethics Committee. The consent of subjects or relatives was obtained according to the Declaration of Helsinki.

Clinical investigations

Patients were clinically evaluated when a steady neurological state had been achieved after interruption of sedative drugs and neuromuscular blocking agents. The clinical signs of CIP were progressive muscle weakness with only minimal movement or complete paralysis in response to speech or painful stimulation and reduced or absent deep tendon reflexes. The clinical state of patients was scored according to a functional disability score including a ranking of the following clinical signs: deep tendon reflexes (0 = normal, 1 = reduced, 2 = absent), muscle weakness (0 = normal muscle force, 2 = mild, 3 = moderate and 4 = severe muscle weakness), muscle atrophy (0 = absent, 1 = present) and muscle fasciculations (0 = absent, 1 = present) as described earlier (Druschky *et al.*, 2001). Organ failure was classified using a modified Goris score (Goris *et al.*, 1985). Multiple organ failure was defined as severe dysfunction of two or more organ systems including the cardiovascular, respiratory, haematological, hepatic, renal and gastrointestinal systems

(Witt *et al.*, 1991; Druschky *et al.*, 2001). Sepsis was defined as the systemic inflammatory response to infection characterized by two or more of the following conditions: (i) temperature $>38^{\circ}\text{C}$ or $<36^{\circ}\text{C}$; (ii) heart rate >90 beats/min; (iii) respiratory rate >20 breaths/min or $\text{PaCO}_2 <4.27$ kPa; (iv) white blood cell count $>12\,000/\text{mm}^3$ or $<4000/\text{mm}^3$; (v) $>10\%$ immature forms (Bone *et al.*, 1992).

Electrophysiological investigations

Nerve conduction studies were performed from the median, peroneal and sural nerves using conventional procedures. Measured parameters were nerve conduction velocity, amplitudes of the compound muscle action potentials (CMAPs) and amplitudes of sensory nerve action potentials (SNAPs). Repetitive stimulation at 3 Hz of the median nerve with recording from the abductor pollicis brevis muscle was done in all patients to screen for neuromuscular transmission defects, for example, due to neuromuscular blocking agents. Concentric needle examinations were performed at multiple insertion points including the abductor pollicis brevis and tibialis anterior muscles. The diagnosis of CIP was established according to the criteria published earlier (Bolton *et al.*, 1984; Zochodne *et al.*, 1987; Bolton, 2005), on the basis of the finding of an acute motor and sensory axonal dysfunction in at least two extremities.

Nerve excitability studies

Studies were performed using a recently described protocol (Kiernan *et al.*, 2000) designed to measure a number of different excitability parameters rapidly. CMAPs were recorded from the abductor pollicis brevis muscle using surface electrodes, with the active electrode at the motor point and the reference electrode on the proximal phalanx. The signal was amplified (gain: 1000, bandwidth: 1.6–2 kHz) and digitized by a data acquisition unit (Micro 1401, Cambridge Electronic Design, Cambridge, UK) using a sampling rate of 10 kHz. Stimulus waveforms generated by a computer were converted to current with a purpose-built isolated linear bipolar constant current stimulator (DS5 prototype, Digitimer Ltd., Welwyn Garden City, UK) (maximum output ± 50 mA). The stimulus currents were applied via non-polarizable electrodes (Red Dot, 3M Health Care, Borken, Germany), with the active electrode over the median nerve at the wrist and reference electrode ~ 10 cm proximal over muscle. Stimulation and recording were controlled by QTRAC software (copyright Institute of Neurology, London) with multiple excitability protocol TRONDCM.

Test current pulses of 0.2–1 ms were applied at 0.8 s intervals and combined with supra-threshold conditioning stimuli or sub-threshold polarizing currents as required. The polarizing, conditioning and test current pulses were all delivered through the same electrodes. The amplitude of the CMAP was measured from baseline to negative peak. For all tracking studies, the target CMAP was set to 40% of maximal CMAP. Skin temperature was monitored over the median nerve close to the site where axonal excitability was tested.

The sequence of recordings was similar to that described previously (Kiernan *et al.*, 2000), except for the determination of the strength–duration relationship and refractory period of transmission. Stimulus–response curves were recorded for test stimuli of 1 ms duration (Fig. 1A). The stimuli were increased in 6% steps, with two responses averaged for each step, until three averages were maximal. A target response was then set at 40% of the maximum and the 1 ms test stimuli adjusted automatically by the computer to maintain this peak CMAP amplitude. The test stimulus amplitude required to excite the target response was regarded as the threshold current for axons recruited at this level

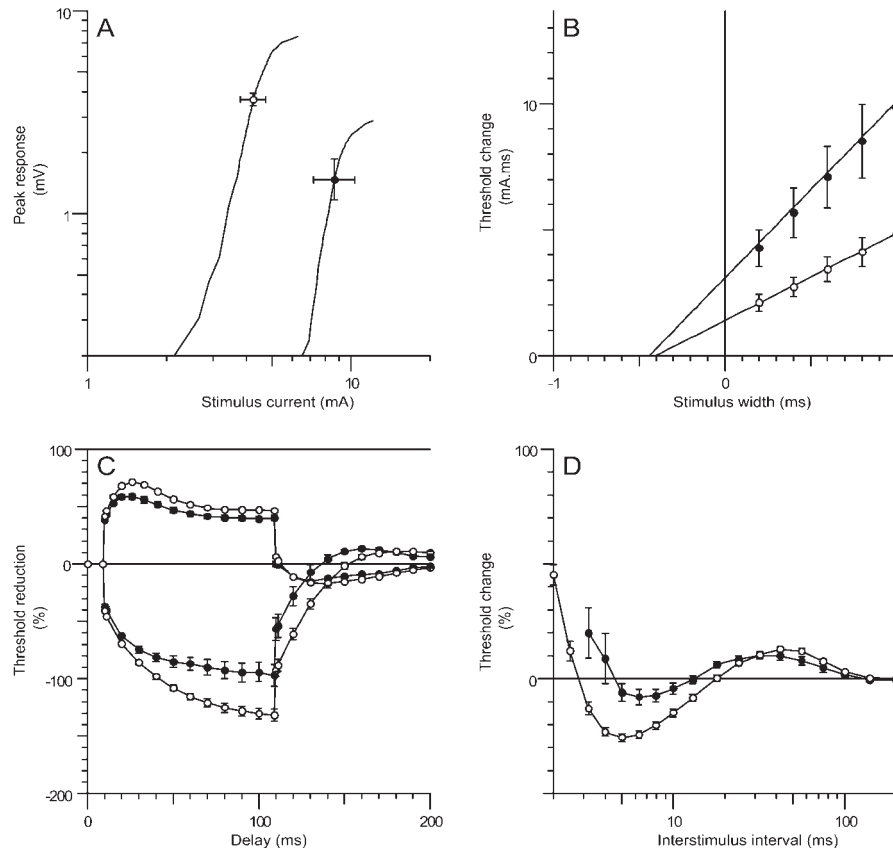


Fig. 1 Nerve excitability changes in CIP. Means and standard error bars for data from 20 normal controls (open circles) and first recordings from 10 patients in intensive care (filled circles). **(A)** Stimulus–response relationship (1 ms stimuli): circles at mean stimulus current for half-maximal peak CMAP. **(B)** Charge–duration relationship, in which intercept on stimulus width axis gives strength–duration time constant, and slope gives rheobase. **(C)** Threshold electrotonus for 100 ms polarizing currents, $\pm 40\%$ of threshold. Responses to depolarizing currents start above the line, and those to hyperpolarizing currents below the line. Standard error bars of many depolarizing responses fall within the circles. **(D)** Recovery cycle following supramaximal stimulation.

of response. ‘Proportional tracking’ was used whereby the change in stimulus amplitude from one trial to the next was made proportional to the ‘error’, or difference between the last response and the target response (Bostock *et al.*, 1998). The slope of the stimulus–response curve was used to set the constant of proportionality and to optimize the tracking efficiency.

The strength–duration relationship for the nerve was measured by comparing the threshold current for a 1 ms test stimulus with the threshold current for stimuli of 0.8, 0.6, 0.4 and 0.2 ms duration. Threshold electrotonus (Bostock *et al.*, 1998) was then measured by recording the changes in threshold induced by 100 ms polarizing currents, set to $\pm 20\%$ and $\pm 40\%$ of the control threshold current. Five stimulus conditions were tested in turn: test stimulus alone (to measure the control threshold current) and test stimulus plus each of the four levels of polarizing current. Threshold was tested at up to 26 time points (maximum separation: 10 ms) before, during and after the 100 ms conditioning currents.

The current–threshold relationship was tested with 1 ms pulses at the end of 200 ms polarizing currents, which were altered in 10% steps from +50% (depolarizing) to –100% (hyperpolarizing) of the control threshold. Stimuli with conditioning currents were alternated with test stimuli alone.

The recovery of excitability following a supramaximal conditioning stimulus was tested at 18 conditioning–test intervals,

decreasing from 200 to 2 ms in geometric progression. Three stimulus conditions were tested in turn: (i) unconditioned 1 ms test stimulus to track the control threshold; (ii) 1 ms supramaximal conditioning stimulus; (iii) conditioning plus test stimuli. The response to (ii) was subtracted online from the response to (iii) before the test CMAP was measured to avoid contamination of the test CMAP by the conditioning one when the conditioning–test interval was short.

The final part of the protocol recorded the refractory period of transmission. Pairs of supramaximal stimuli were delivered at conditioning–test intervals of 10, 7, 5, 4, 3, 2 and 1 ms to investigate the time course of any changes in impulse transmission (Kuwabara *et al.*, 2003). The intensity of the conditioning stimulus was 20% above that required for producing a maximal CMAP, and the second stimulus was set 50% higher than the first. The conditioned CMAP was measured after the response to the supramaximal conditioning stimulus had been subtracted. The amplitude of the conditioned CMAP was normalized to the unconditioned value.

Laboratory examinations

Laboratory examinations were performed either before or after, but always within 90 min of the electrophysiological investigations, and included serum levels of sodium, potassium, chloride, glucose,

Table 1 Clinical and laboratory characteristics of patients with CIP and normal control subjects

	Normal control subjects	CIP-1	CIP-2	P-value	
				ANOVA	t-test Wilcoxon ^w
Numbers of patients	20	10	8		
Age (years)	58.0 ± 3.2	62.2 ± 3.72	60.75 ± 4.71	0.71	
Male/female	7/13	3/7	3/5		
FDS	0	3 (range: 1–6)	3 (range: 1–4)		0.71 ^w
MOF	0	2 (range: 1–3)	2.5 (range: 2–4)		0.41 ^w
Sepsis	0	5	5		
Potassium	4.01 ± 0.08	4.32 ± 0.15	4.15 ± 0.11	0.11	0.17
Creatinine	73.7 ± 3.0	120.6 ± 34.08	94.25 ± 31.47	0.22	0.49
CRP	3.3 ± 0.3	121.3 ± 26.01	75.0 ± 26.5	0.00001 ^{*****}	0.58
pO ₂	9.91 ± 0.23 (n = 10)	11.01 ± 7.8	11.32 ± 0.85	0.3	0.78
pCO ₂	4.44 ± 0.21 (n = 10)	5.84 ± 0.34	5.68 ± 0.35	0.005 ^{**}	0.77
pH	7.44 ± 0.01 (n = 10)	7.42 ± 0.01	7.44 ± 0.01	0.39	0.95
BE	−0.66 ± 0.66 (n = 10)	2.35 ± 0.87	3.94 ± 1.21	0.005 ^{**}	0.67
Bicarbonate	22.32 ± 0.8 (n = 10)	27.15 ± 0.82	29.24 ± 1.83	0.001 ^{***}	0.22

ANOVA (analysis of variance) compares all three groups, whereas paired t-test or Wilcoxon matched pairs test compares measurements on CIP patients. Values are given as mean ± SEM; values of FDS and MOF are given as median and range (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, *****P < 0.00001). FDS = functional disability score; MOF = multiple organ failure; CRP = C-reactive protein.

lactate, creatinine, C-reactive protein, arterial pO₂, pCO₂, bicarbonate, base excess (BE) and pH.

Data analysis

The following excitability parameters were derived from each recoding: resting current–threshold slope (the slope of the current–threshold relationship, calculated from the polarizing currents between −10 and +10% of resting threshold); minimum current–threshold slope (the minimum slope, calculated by fitting a straight line to each three adjacent points in turn); relative refractory period (calculated from the recovery cycle data as the first intercept on the x-axis); superexcitability (the interpolated threshold change at an interstimulus interval of 7 ms N.B.; the normal measure of superexcitability, that is, the minimum mean of three adjacent points, was inappropriate for CIP patients, since in some cases there was no minimum); subexcitability (the maximum mean of three adjacent points of the recovery cycle); the *threshold electrotonus* parameters TE_d[10–20 ms], TE_d[90–100 ms], TE_h[90–100 ms] [mean threshold reductions between the specified latencies for the 40% depolarizing (TE_d) and hyperpolarizing (TE_h) currents] and the refractory period of transmission (calculated as the interval between the conditioning and test stimulus that causes a drop of the amplitude of the conditioned CMAP of 50% compared with the amplitude of the CMAP induced by the conditioning stimulus). Data were compared using unpaired two-tailed *t*-tests. Relative refractory period measurements were compensated for temperature using the relationship found in normal control subjects (Kiernan et al., 2000).

Results

Patients' and control subjects' characteristics, including mean age, gender and clinical and laboratory characteristics are summarized in Table 1. All patients fulfilled the electrophysiological criteria for CIP, with decreased amplitudes of CMAPs in motor nerve conduction studies, fibrillation potentials and positive sharp waves in electromyography

and severe depression (*n* = 3) or absence (*n* = 7) of SNAP amplitudes (in the absence of tissue oedema). No patient showed clinical or electrophysiological signs of neuro-muscular transmission defect. All patients suffered multiple organ failure or sepsis. In four patients these two entities were combined. To determine which axonal properties are significantly affected in CIP, the first recordings from each patient (i.e. the 10 CIP-1 observations in Table 1) were compared with the data from the similarly aged controls. When possible, a second examination (CIP-2) was made ~2 weeks after the first, in case the development or progression of neuropathy could be related to the earlier measurements. In the event, the second examinations (average 15.1 ± 4.7 days later, mean ± SE) did not differ significantly from the first, either in clinical and laboratory data (Table 1) or in the nerve excitability measurements. For the purposes of determining the mechanisms responsible for the changes in excitability parameters, we combined the data from both sets of patient recordings (i.e. the 10 CIP-1 and 8 CIP-2 observations listed in Table 1).

Nerve excitability data for the CIP patients (first recordings) and normal control subjects are compared in Figs 1 and 2. Thresholds for a half-maximal response (1 ms stimulus) were higher in the patients (CIP-1: 10.0 ± 1.8 mA, mean ± SE, *n* = 10; NC: 4.9 ± 0.7, *n* = 20, *P* < 0.01) and the maximal patient CMAPs were significantly smaller (CIP-1: 3.7 ± 0.9 mV; NC: 8.0 ± 0.6 mV; *P* < 0.001) (Fig. 1A). The most significant abnormalities in the CIP patients were a 'fanning-in' of threshold electrotonus (i.e. a reduction in the absolute size of the threshold changes produced by polarizing currents) (Fig. 1C) and a reduction in superexcitability (Fig. 1D), both of which changes occur on membrane depolarization (Kiernan and Bostock, 2000). Thus the early depolarizing electrotonus, from 10 to 20 ms after a 40% of threshold depolarizing current (TE_d[10–20 ms]) was

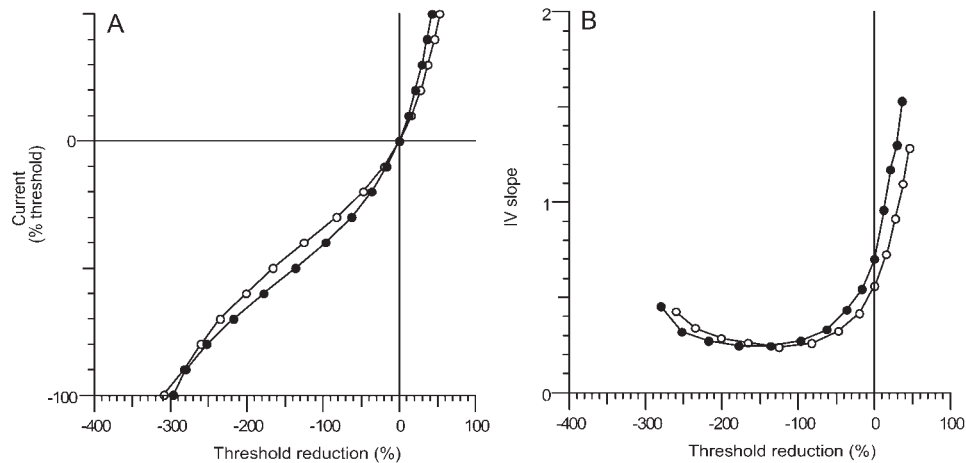


Fig. 2 Mean current–threshold relationship (**A**) and its slope (**B**) for patients (filled circles) and normal controls (open circles). The slope of the current–threshold relationship is the threshold analogue of conductance, and increases on depolarization (to the right of vertical line), owing to activation of outwardly rectifying potassium channels, and also on extreme hyperpolarization (far left), owing to activation of inwardly rectifying I_H . Patient curve is displaced to the left, indicating that rectifying channels are relatively depolarized.

reduced from $70.1 \pm 1.4\%$ to $58.3 \pm 2.4\%$ ($P < 0.001$) and depolarizing electrotonus at 90–100 ms (TED[90–100 ms]) was reduced from $46.6 \pm 1.2\%$ to $39.6 \pm 2.2\%$ ($P < 0.01$). Hyperpolarizing electrotonus at 90–100ms (TEH[90–100 ms]) was reduced from $-131.0 \pm 4.9\%$ to $-95.7 \pm 8.8\%$ ($P < 0.001$). Superexcitability at 7 ms was reduced by two-thirds, from $-22.3 \pm 1.6\%$ to $-7.6 \pm 3.1\%$ ($P \approx 0.0001$). The changes in most other excitability parameters were also consistent with depolarization (Kiernan and Bostock, 2000). The relative refractory period was increased from $2.8 \pm 0.09\%$ to $3.9 \pm 0.5\%$ ($P < 0.01$), and the strength–duration time constants were longer in the patients, but not significantly so (CIP-1: 0.44 ± 0.05 ms; NC: 0.41 ± 0.02 ms; $P = 0.44$) (Fig. 1B). The current–threshold relationship, the threshold analogue of the current–voltage (I/V) relationship, is shown in Fig. 2A, and the slope of this curve, which is the threshold analogue of conductance, is plotted in Fig. 2B. The resting slope was increased in the patients (CIP-1: 0.93 ± 0.22 ; NC: 0.57 ± 0.02 ; $P < 0.05$) consistent with membrane depolarization (Kiernan and Bostock, 2000). Perhaps the clearest evidence for depolarization is that the ‘I/V slope’ in Fig. 2B is shifted to the left in the patients; the threshold analogue of a shift of the conductance changes to more negative potentials [or of the resting potential (E_r) to a more depolarized value, at which the slope is higher].

Although there was overlap between the control and CIP groups for all excitability parameters, 7 of the 18 measurements of superexcitability and 5 of the 18 measurements of TED[10–20 ms] in CIP patients fell outside the 95% confidence limits for a healthy subject.

Nerve excitability changes and renal failure

In previous excitability studies it has been found that the phase of late subexcitability in the recovery cycle can

provide evidence about the mechanism of membrane depolarization, since it is generated by outward potassium currents and depends on the difference between the membrane potential and the potassium equilibrium potential (E_k). Thus, depolarization by applied currents (without changing extracellular potassium) increases late subexcitability (Kiernan and Bostock, 2000), depolarization by hyperkalaemia reduces late subexcitability (Kiernan *et al.*, 2002b) and depolarization by acute ischaemia occurs with little or no change in late subexcitability (Kiernan and Bostock, 2000). In the 18 recordings from patients with CIP, subexcitability was unchanged on average (CIP-All: 10.7 ± 1.6 ; NC: 12.2 ± 1.1 ; $P = 0.41$), suggesting that depolarization was accompanied by a small increase in extracellular potassium, as occurs in acute ischaemia. However, we found that there was a significant correlation between late subexcitability and creatinine ($R = 0.67$, $P < 0.005$), and when the patient recordings were subdivided into 7 with evidence of renal failure (CIP_{RF+}, creatinine $> 100 \mu\text{M}$) and 11 without (CIP_{RF-}, creatinine $< 100 \mu\text{M}$), there was a significant reduction of late subexcitability in the recordings from patients with renal failure, but not in those without (CIP_{RF+}: 7.9 ± 1.2 , $P < 0.05$; CIP_{RF-}: 13.1 ± 0.9 , $P = 0.59$). This provides evidence that intraneural potassium was high in the patients with renal failure. In contrast, superexcitability and electrotonus markers for membrane depolarization were not significantly different between the patients with renal failure and those without (Fig. 3), so that potassium was unlikely to be responsible for the depolarization in the majority of patients without renal failure.

The close relationship between membrane potential and extracellular potassium levels in the patients with renal failure is shown more directly by plotting the potential-sensitive excitability parameters superexcitability and TED[10–20 ms] against serum potassium in Fig. 4. All the ellipses, in normal

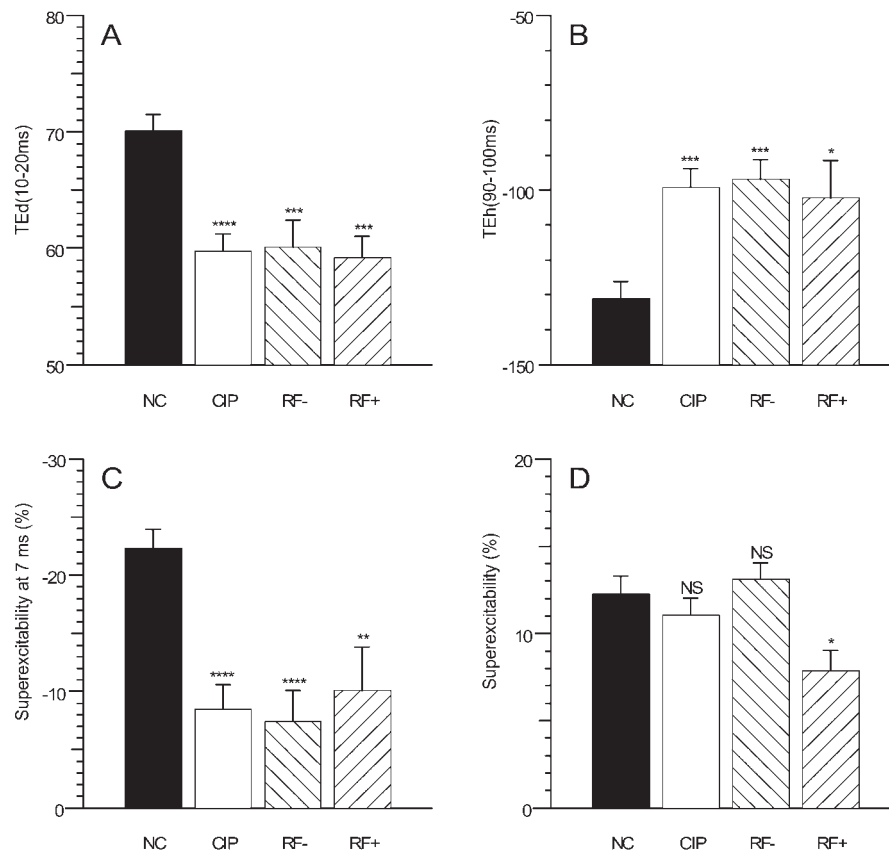


Fig. 3 Excitability parameters and their relationship to renal failure in CIP. Electrotonus and recovery cycle parameters compared for 20 normal control (NC) and 18 CIP recordings, as a whole and separated into 7 with renal failure (RF+) and 11 without (RF-). Values plotted are means with standard error bars. Labels on columns 2–4 indicate probabilities that values are drawn from the same population as NC. NS = not significant ($P > 0.05$), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

controls as well as both patient groups, show a similar slope, corresponding to membrane depolarization with an increase in serum potassium, but only in the patients with renal failure are the correlations significant.

Nerve excitability and chronic respiratory acidosis

Table 1 shows that whereas pH was normal in the patients, serum levels of $p\text{CO}_2$, BE, bicarbonate and corrected anion gap were abnormally high. This indicates that the patients were suffering from chronic respiratory acidosis, combined with metabolic alkalosis. When each of these levels was tested for correlation with the excitability parameters within the 18 patient recordings, only bicarbonate and BE showed significant correlations with multiple excitability indices of membrane potential. Excitability indices of membrane potential correlated with serum bicarbonate levels, but only in the patients without renal failure. Figure 5 is analogous to Fig. 4, with bicarbonate replacing potassium as the abscissa, and shows a complementary relationship: whereas in patients with kidney failure membrane potential was strongly related to serum potassium, in patients with

preserved kidney function membrane potential is more closely related to serum bicarbonate, and therefore to the chronic respiratory acidosis.

Nerve excitability changes and other factors

We have seen evidence that the membrane depolarization in CIP patients is related to extracellular potassium in patients with renal failure and to respiratory acidosis in those without. Here, we test whether these two factors together can account for the membrane depolarization, or whether other factors, such as the unknown toxic factors previously hypothesized, are likely to be involved. Table 2 shows the fraction of the variance in excitability parameters accounted for by correlation with serum potassium, serum bicarbonate and skin temperature, and by multiple correlations with all three variables combined. Temperature is included, since relative refractory period is known to be particularly sensitive to temperature. The analysis is performed for the combined group of patients and controls, as well as for the separate patient and control groups, since we are interested in factors accounting for the differences between patients and controls,

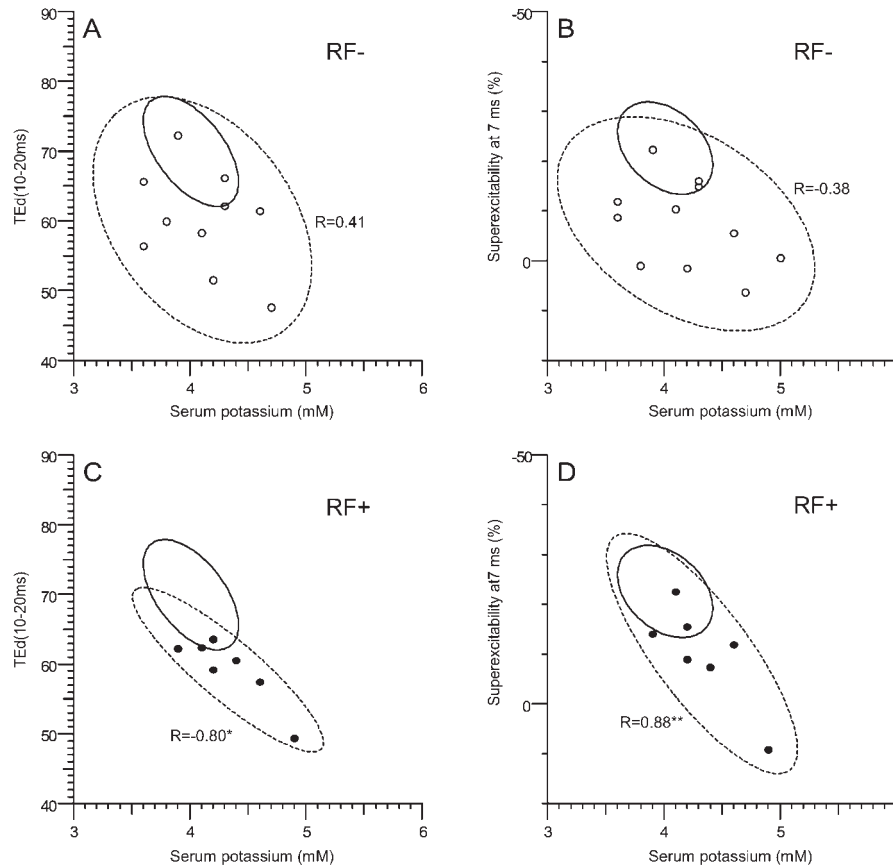


Fig. 4 Excitability parameters related to serum potassium and renal failure. In each plot, circles represent subset of CIP recordings (filled circles: with renal failure, open circles: without renal failure), dashed ellipses are 95% confidence limits for the group, and R -values are correlation coefficients (* $P < 0.05$, ** $P < 0.01$). Solid ellipses indicate mean \pm standard deviation of normal controls ($\sim 68\%$ limits).

as well as the variation within the patient group. Consistent with the results reported above, among the patients early depolarizing threshold electrotonus is significantly correlated with serum potassium in those with renal failure, and with bicarbonate in those without renal failure, but the combination of potassium and bicarbonate accounts for over 50% of the variance, and for almost 70% of the variance in the combined group of patients and controls. For the other strongly potential-dependent excitability parameters, such as superexcitability, a high proportion of the variance is also explained by correlation with the same two variables. These figures indicate that any independent factor can only account for a relatively small fraction of the changes in membrane potential. For example, C-reactive protein, an indicator of acute inflammation, might have been expected to correlate with neurotoxic factors affecting nerve excitability (Druschky *et al.*, 2001). However, there was no significant correlation between C-reactive protein levels and any of the excitability parameters in the patients.

Discussion

CIP was first described in 1984 (Bolton *et al.*, 1984) and has subsequently become widely recognized as a common

polyneuropathy, occurring in 50–70% of patients who are critically ill or injured (Berek *et al.*, 1996; Leijten *et al.*, 1996; Hund, 2001). Such patients have a syndrome now termed the systemic inflammatory response syndrome (Bolton, 1996). This syndrome occurs in response to both infection and several forms of trauma, including major surgery and has been attributed to humoral neurotoxic factors (Druschky *et al.*, 2001). After the development of systemic inflammatory response syndrome in a critical care unit, the earliest nervous system manifestation is encephalopathy, which can gradually progress to deep coma. If the syndrome responds to treatment (antibiotics, surgical drainage of an infected focus, supportive measures) the encephalopathy usually improves rapidly, but at this time difficulty in weaning from the ventilator will be noted. Studies have shown that the most common neuromuscular cause for such weaning difficulty is CIP (Zochodne *et al.*, 1987; Lemaire, 1993). However, clinical signs of polyneuropathy are present in only half of these patients. Hence electrophysiological studies are necessary for establishing the diagnosis. The morphological features of CIP have been revealed through biopsy of peripheral nerve and muscle and a comprehensive autopsy study (Zochodne *et al.*, 1987). These studies indicate primary distal axonal degeneration of

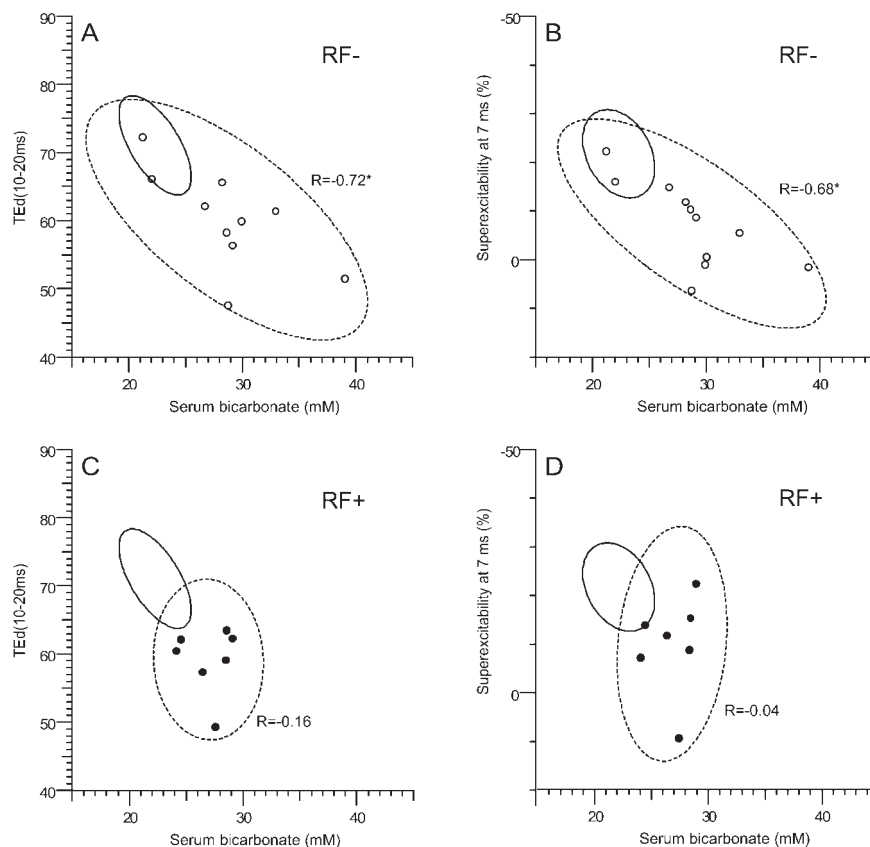


Fig. 5 Excitability parameters related to serum bicarbonate and renal failure, plotted as in Fig. 4.

peripheral motor and sensory nerve fibres, but up to now the aetiology of the axonal neuropathy has remained unclear.

The current diagnostic criteria for CIP require patient history, clinical data and electrophysiological evidence of an axonal motor and sensory polyneuropathy (Bolton, 2005). The differential diagnosis of CIP includes other neuromuscular transmission defects and myopathies, of which the most important one is critical illness myopathy. According to neurophysiological (Rich *et al.*, 1997) and biopsy (Latronico *et al.*, 1996) studies, myopathies occur much more frequently during critical illness than previously recognized. Three main types, often termed together as acute quadriplegic myopathy or acute myopathy of intensive care, have been identified: a diffuse non-necrotizing myopathy ('critical illness myopathy'), myopathy with selective loss of thick (myosin) filaments and the acute necrotizing myopathy of intensive care (Hund, 1999). Differentiating CIP from critical illness myopathy is difficult, and sometimes these two syndromes can occur simultaneously. Both entities show low CMAP amplitudes in motor nerve conduction studies. Furthermore, needle electromyography is of little use in distinguishing between myopathy and polyneuropathy, because fibrillation potentials and positive sharp waves as well as decreased recruitment can be present in CIP and in critical illness myopathy. Stimulation of muscle directly and indirectly (by stimulating the nerves to the muscle) may

provide some more information, but a reliable diagnosis can only be achieved after muscle and/or nerve biopsy (Sander *et al.*, 2002; Bolton, 2005; Latronico *et al.*, 2005). Depression or absence of SNAP amplitudes (in the absence of severe tissue oedema) is a strong indicator of CIP, since SNAP amplitudes are normal in pure critical illness myopathy. In the present study, all patients showed abnormally small SNAPs, and in 7 out of the 10 patients SNAPs were unrecordable, although the recording conditions were good. This finding, coupled with the fact that none of our patients had a previously documented polyneuropathy or suffered from a systemic disease known to be strongly associated with polyneuropathy, indicates the presence of CIP. This does not exclude the possibility that an accompanying critical illness myopathy may have contributed to the patients' weakness. However, since nerve excitability tests study nerve alterations at the site of stimulation, and do not depend on the state of the muscle (provided CMAPs are recordable), the possible simultaneous occurrence of critical illness myopathy could not influence our measurements of nerve excitability.

Our results provide evidence that axons are depolarized in the CIP patients, and the excitability measurements also provide some interesting clues as to the likely mechanisms underlying membrane depolarization. In view of the previous evidence linking renal failure to depolarization via

Table 2 Coefficients of determination (R^2) for regression of excitability parameters on three environmental factors, and for multiple regressions on all three factors combined

	Serum potassium	Serum bicarbonate	Skin temperature	Three variables
Superexcitability				
NC	0.186	0.088	0.003	0.33
CIP	0.211	0.296*	0.005	0.462**
NC + CIP	0.265***	0.494***	0.006	0.615****
TEd (10–20 ms)				
NC	0.311*	0.398*	0.162	0.622*
CIP	0.245*	0.298*	0.031	0.527**
NC + CIP	0.313***	0.552****	0.046	0.696****
TEd (90–100 ms)				
NC	0.151	0.074	0.079	0.154
CIP	0.173	0.195	0.015	0.354*
NC + CIP	0.221**	0.356**	0.028	0.456**
TEh (90–100 ms)				
NC	0.355**	0.098	0.167	0.736**
CIP	0.247*	0.07	0.009	0.314*
NC + CIP	0.339***	0.242**	0.007	0.461***
RRP				
NC	0.181	0.014	0.048	0.473
CIP	0.274	0.004	0.452**	0.594**
NC + CIP	0.269**	0.122	0.261**	0.602****

NC = normal control, CIP = patients with critical illness polyneuropathy, RRP = relative refractory period, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, ***** $P < 0.00001$.

hyperkalaemia (Kiernan *et al.*, 2002b; Krishnan *et al.*, 2005), and the high frequency of renal failure in critical care patients, there were strong *a priori* reasons for supposing that hyperkalaemia should be involved. However, there was little evidence from the mean serum potassium level (4.24 mM, $n = 18$) that hyperkalaemia could account for an appreciable degree of membrane depolarization. That leaves us with the question of what was the level of potassium in the tissues, especially in the vicinity of the axons. The best indicator of endoneurial potassium from nerve excitability measurements is the phase of late subexcitability in the recovery cycle. This occurs reliably in normal motor axons, owing to the activation of slow potassium channels during the action potential (Schwarz *et al.*, 1995). These channels inactivate slowly, so that they normally generate a late hyperpolarizing after-potential and period of subexcitability, since the potassium equilibrium potential (E_k) is more negative than the resting potential (E_r). The subexcitability therefore depends on the difference between these quantities: $E_r - E_k$. Thus, subexcitability increases when an axon is depolarized by applied currents (Kiernan and Bostock, 2000), which make E_r more positive without changing E_k , and subexcitability decreases when an axon is depolarized by extracellular potassium, which makes E_k more positive. In the CIP patients subexcitability was inversely correlated with creatinine, suggesting that renal insufficiency resulted in raised intraneurial potassium, even though serum potassium remained relatively normal. (A direct effect of creatinine on

the slow potassium channels can be excluded, since no such relationship was seen in the earlier study of patients with renal failure.) We therefore hypothesize that in the patients with renal insufficiency raised levels of endoneurial potassium contribute to the membrane depolarization and hence to the pathogenesis of CIP. In support of this, in spite of the small range of values, there was a significant correlation between serum potassium and excitability parameters in the patients with renal insufficiency (Fig. 4) (*see also* Table 2).

In patients without renal failure superexcitability was reduced to the same degree as in patients with additional renal failure, whereas late subexcitability in the recovery cycle was unchanged compared with normal control subjects. This finding provides evidence of another mechanism of membrane depolarization than in patients with additional acute renal failure. Similar changes of superexcitability and unchanged late subexcitability have been reported previously in acute ischaemia (Kiernan and Bostock, 2000). Furthermore, significant correlations between superexcitability or TEd[10–20 ms] and the measured serum bicarbonate and BE were present in this patient group (Fig. 5). Although pH was within normal limits in all patients, pCO_2 , bicarbonate, BE and corrected anion gap were moderately to abnormally raised. These findings suggest a mixed acid–base disorder consisting of compensated respiratory acidosis and metabolic alkalosis in these patients. The question arises of whether the increase in pCO_2 , by causing intracellular acidification, was thereby somehow responsible for the membrane depolarization. This seems unlikely, since a high concentration of pCO_2 has been found to cause a ‘fanning-out’, rather than a ‘fanning-in’ of electrotonus, by blocking potassium channels (Grafe *et al.*, 1994). A more plausible interpretation is that the reason why membrane depolarization is related to bicarbonate and pCO_2 is that both depolarization and respiratory acidosis are caused by poor oxygenation of the tissues. This was not evident from the recorded pO_2 values, because pO_2 was maintained by artificial ventilation and/or oxygen treatment. Furthermore, all patients of this study suffered from systemic hypotension during their stay in intensive care, and were therefore treated with noradrenalin to maintain a sufficient systemic blood pressure. The combination of these factors probably leads to a further hypoperfusion of the small capillaries of the nerves and via a local lack of oxygen to an accumulation of acidic metabolites.

The degree of membrane depolarization seen in the axons of the CIP patients was similar to that reported previously in patients with chronic renal failure (Kiernan *et al.*, 2002b). It was hypothesized that the chronic membrane depolarization might be instrumental in causing the axonal damage of uraemic neuropathy, by interfering with potential-dependent mechanisms of ionic homeostasis (e.g. Na^+/Ca^{2+} exchange) essential for cellular viability (Kiernan *et al.*, 2002b; Bostock *et al.*, 2004). On this basis, it seems reasonable to propose that in the critically ill patients the chronic axonal membrane depolarization due to hyperkalaemia and/or

hypoperfusion may also be involved in the pathophysiology of the neuropathy. This would be consistent with the findings of Latronico *et al.* (1996) that most sensory nerves in early biopsies (median: 15 days sepsis) looked normal, despite having reduced SNAPs, while in late biopsies (56 days) electrophysiological and histological findings were concordant. They suggested that early impairment of axonal transport and transmembrane potential preceded structural damage.

In conclusion, we have found evidence that in these CIP patients peripheral nerves are depolarized, and the correlations with serum factors suggest that this membrane depolarization is related to endoneurial hyperkalaemia and/or hypoxia. While other mechanisms of depolarization may well be involved, the degree to which potential-sensitive nerve excitability indices are related to serum potassium and bicarbonate suggests that other factors, independent of potassium and acid–base balance, are likely to be of relatively minor significance.

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References

- Berek K, Margreiter J, Willeit J, Berek A, Schmutzhard E, Mutz NJ. Polyneuropathies in critically ill patients: a prospective evaluation. *Intensive Care Med* 1996; 22: 849–55.
- Bolton CF. Sepsis and the systemic inflammatory response syndrome: neuromuscular manifestations. *Crit Care Med* 1996; 24: 1408–16.
- Bolton CF. Neuromuscular manifestations of critical illness. *Muscle Nerve* 2005; 32: 140–63.
- Bolton CF, Gilbert JJ, Hahn AF, Sibbald WJ. Polyneuropathy in critically ill patients. *J Neurol Neurosurg Psychiatry* 1984; 47: 1223–31.
- Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992; 101: 1644–55.
- Bostock H, Cikurel K, Burke D. Threshold tracking techniques in the study of human peripheral nerve. *Muscle Nerve* 1998; 21: 137–58.
- Bostock H, Walters RJ, Andersen KV, Murray NM, Taube D, Kiernan MC. Has potassium been prematurely discarded as a contributing factor to the development of uraemic neuropathy? *Nephrol Dial Transplant* 2004; 19: 1054–7.
- Druschky A, Herkert M, Radespiel-Troger M, Druschky K, Hund E, Becker CM, et al. Critical illness polyneuropathy: clinical findings and cell culture assay of neurotoxicity assessed by a prospective study. *Intensive Care Med* 2001; 27: 686–93.
- Goris RJ, te Boekhorst TP, Nuytinck JK, Gimbrere JS. Multiple-organ failure. Generalized autodestructive inflammation? *Arch Surg* 1985; 120: 1109–15.
- Grafe P, Bostock H, Schneider U. The effects of hyperglycaemic hypoxia on rectification in rat dorsal root axons. *J Physiol* 1994; 480: 297–307.
- Hund E. Myopathy in critically ill patients. *Crit Care Med* 1999; 27: 2544–7.
- Hund E. Critical illness polyneuropathy. *Curr Opin Neurol* 2001; 14: 649–53.
- Kiernan MC, Bostock H. Effects of membrane polarization and ischaemia on the excitability properties of human motor axons. *Brain* 2000; 123: 2542–51.
- Kiernan MC, Burke D, Andersen KV, Bostock H. Multiple measures of axonal excitability: a new approach in clinical testing. *Muscle Nerve* 2000; 23: 399–409.
- Kiernan MC, Guglielmi JM, Kaji R, Murray NM, Bostock H. Evidence for axonal membrane hyperpolarization in multifocal motor neuropathy with conduction block. *Brain* 2002a; 125: 664–75.
- Kiernan MC, Walters RJ, Andersen KV, Taube D, Murray NM, Bostock H. Nerve excitability changes in chronic renal failure indicate membrane depolarization due to hyperkalaemia. *Brain* 2002b; 125: 1366–78.
- Krishnan AV, Phoon RK, Pussell BA, Charlesworth JA, Bostock H, Kiernan MC. Altered motor nerve excitability in end-stage kidney disease. *Brain* 2005; 128: 2164–74.
- Kuwabara S, Bostock H, Ogawara K, Sung JY, Kanai K, Mori M, et al. The refractory period of transmission is impaired in axonal Guillain-Barre syndrome. *Muscle Nerve* 2003; 28: 683–9.
- Latronico N, Fenzi F, Recupero D, Guarneri B, Tomelleri G, Tonin P, et al. Critical illness myopathy and neuropathy. *Lancet* 1996; 347: 1579–82.
- Latronico N, Peli E, Botteri Latronico M. Critical illness myopathy and neuropathy. *Curr Opin Crit Care* 2005; 11: 126–32.
- Leijten FS, De Weerd AW, Poortvliet DC, De Ridder VA, Ulrich C, Harink-De Weerd JE. Critical illness polyneuropathy in multiple organ dysfunction syndrome and weaning from the ventilator. *Intensive Care Med* 1996; 22: 856–61.
- Lemaire F. Difficult weaning. *Intensive Care Med* 1993; 19 (Suppl 2): S69–73.
- Rich MM, Bird SJ, Raps EC, McCluskey LF, Teener JW. Direct muscle stimulation in acute quadriplegic myopathy. *Muscle Nerve* 1997; 20: 665–73.
- Sander HW, Golden M, Danon MJ. Quadriplegic areflexic ICU illness: selective thick filament loss and normal nerve histology. *Muscle Nerve* 2002; 26: 499–505.
- Schwarz JR, Reid G, Bostock H. Action potentials and membrane currents in the human node of Ranvier. *Pflugers Arch* 1995; 430: 283–92.
- Witt NJ, Zochodne DW, Bolton CF, Grand'Maison F, Wells G, Young GB, et al. Peripheral nerve function in sepsis and multiple organ failure. *Chest* 1991; 99: 176–84.
- Zochodne DW, Bolton CF, Wells GA, Gilbert JJ, Hahn AF, Brown JD, et al. Critical illness polyneuropathy. A complication of sepsis and multiple organ failure. *Brain* 1987; 110: 819–41.